# Troubleshooting a Cloning Experiment

Prepared by Ms Alex Aitken

Often, suitable controls will pinpoint the origin of the problem.

No colonies

- To confirm that the ligase and ligase buffer are functional, repeat the experiment with an uncut plasmid and a single cut plasmid with and without T4 DNA Ligase.
- To confirm competent cell viability transform an uncut plasmid.
- In all instances repeat with fresh buffer as the ATP or DTT may have degraded.
- Purify the DNA as the presence of NaCl or EDTA will reduce ligation efficiency – gel extract the DNA to ensure complete removal of such inhibitors.
- Confirm there is a 5´ phosphate on either the insert or plasmid – using freshly produced DNA products is the only way to control for this.
- Ligations can produce only linear DNA because the DNA concentration is too high. Keep the total DNA concentration between 1-10 µg/ml.
- Confirm the correct antibiotic has been used on agar plates.

Too many colonies

- The agar plates should be no more than 1 week old.
- Vector has self ligated – dephosphorylate with fresh enzyme.
- Competent cells are mutants – check RecA- status.

Too few colonies

- This might be the way it is – if insert or vector DNA has folded and obscured the ends there is little which can be done.
- Increase the overall amount of DNA as well as adjusting the ratios.